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Degradation of habitat disrupts plant–pollinator interactions for a rare self-compatible plant

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Abstract Habitat destruction has immediate consequences on biodiversity, whereas the effects of habitat degradation are slower and more subtle. Habitat quality and structure influence reproduction in rare plant species because changes in the local environment can disrupt sensitive plant–pollinator interactions. We used the self-compatible rare species *Synthyris bullii* to examine pollination and reproduction in response to woody encroachment, a type of degradation that occurs in prairies and savannas in the absence of fire. Additionally, we determined if autonomous selfing occurred more frequently than pollinator-mediated fertilization in degraded habitats. Infructescences from populations in open, semi-shaded, shaded habitats (i.e., different levels of encroachment) were collected to assess reproductive output (e.g., fruit/seed set) and fitness (i.e., germination). In addition, a pollinator exclusion treatment was conducted in these habitats to estimate pollen quantity (i.e., stigma pollen load). Pollinators contributed 32–57 % of pollen loads on average. We observed a significant increase in reproductive output associated with the pollinator treatment, even when the relative pollen contribution was small. Further, fruit and seed set were negatively affected by pollinator exclusion regardless of habitat type. We found evidence that pollen quantity/quality was lower in shaded habitats, which also played a role in lower fruit/seed set and germination compared to other habitats. Autonomous selfing does not occur at a sufficiently high rate, even in shaded habitats, to compensate for pollinator absence. As habitats degrade, reduced pollen quantity/quality and low autonomous selfing rates may contribute to the loss of rare species.

Keywords Habitat degradation · Pollen quality · Pollen quantity · Rare plants · Selfing

Introduction

Habitat destruction is recognized as the leading cause of species decline and extinction (Wilcove et al. 1998; Pimm and Raven 2000). However, surviving populations in remnant patches may be threatened over time as a result of habitat degradation, primarily because species adapted to particular habitat conditions may be unable to tolerate changes to the quality and structure of their environment (McKinney and Lockwood 1999; Malcolm et al. 2006; Maskell et al. 2010).

The effects of habitat loss and degradation can be further amplified through the disruption of species interactions, especially mutualisms (Dunn et al. 2009).

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Rare plant species that depend on animals for pollination are particularly vulnerable to habitat changes as reproduction and recruitment are impeded by the availability and ability of pollinators to visit plants in remnant patches (Kearns and Inouye 1997; Kearns et al. 1998; Wilcock and Neiland 2002; Dunn et al. 2009; Potts et al. 2010). Some plant populations can be sustained in the short- term through strategies such as autonomous self-fertilization and clonal reproduction (Honnay and Bossuyt 2005; Van Kleunen et al. 2007; Pauw and Bond 2011). However, the long-term viability of these populations may be compromised by decreased genetic diversity limiting a population's ability to respond to changing habitat conditions (Honnay and Bossuyt 2005; Honnay and Jacquemyn 2007). This is of particular concern for endangered species because a frequent condition of their rarity is their tolerance for only a narrow range of environmental conditions (Rabinowitz 1981).

Much research on habitat loss and plant–pollinator relationships has been devoted to fragmentation, patch size, and other correlates for habitat quality with fewer studies examining drivers of habitat degradation, such as fire and management (Potts et al. 2010). In North America, most prairies and savannas have been destroyed by conversion to agricultural lands and the remaining remnants suffer from increased habitat degradation due to disruption of historic fire patterns that leads to encroachment by early successional woody species (Leach and Givnish 1996; Briggs et al. 2002; Van Auken 2009). Indeed, research has shown a decrease in species diversity associated with invasion by woody species in grasslands and savannas (Rejmánek and Rosén 1988; Ratajczak et al. 2012). Herbaceous species that characterize these communities experience additional constraints as a result of the ability of woody species to (1) compete directly for abiotic resources (Wilson 1993; Van Auken and Bush 1997), and (2) alter the abiotic environment (e.g., soil chemistry, canopy structure) in a way that is disadvantageous to herbaceous species (Wilson 1993; Kennedy and Sousa 2006). These changes have direct consequences for rare prairie species because environmental changes and competition for resources may limit the plants' ability for successful reproduction and recruitment (Shivanna and Tandon 2014).

Shading from woody encroachment also affects access to biotic resources by changing foraging behavior of insect pollinators. As ectothermic organisms, insects are particularly sensitive to microclimatic changes and may avoid shaded areas where temperatures are cooler (Herrera 1995; Culley 2002; Cortes-Palomec and Ballad 2006; Kilkenny and Galloway 2008). Insect avoidance of shading may result in low pollinator visitation for shaded plants, and subsequently reduced seed set (McKinney and Goodell 2010). This shade-avoidance behavior may be especially true for pollinators that occur in prairies and are presumably adapted to forage in high light environments. Due to the absence of pollinators, self-compatibility may also be favored in areas where woody encroachment has deterred insect pollinators. Studies that examine variation in mating systems across species' ranges (i.e., across a gradient of pollinator availability and environmental conditions) often show that selfing rates increase in response to increased physiological stress and pollinator limitation (Busch 2005; Moeller 2006; Moeller et al. 2012).

This study aims to test whether woody encroachment interferes with pollination and reproduction, thereby compromising the viability of rare plant populations. Our specific objective was to examine the effects of shade from woody encroachment on the reproductive processes of a rare herbaceous species, *Synthyris bullii*, which occurs in open prairie–savanna habitats. Specifically, our study aims to determine (1) how reproduction and fitness in *S. bullii* are affected by pollen quantity, (2) if pollen quantity, reproductive output (e.g., fruit/seed set), and fitness (i.e., seed germination) are negatively impacted in areas of woody encroachment, and (3) if autonomous selfing is more likely to occur in plants growing in encroached habitats compared to those found in open ones.

Materials and methods

Study species

Synthyris bullii (Eaton) A. Heller (Plantaginaceae; Kittentail; synonym *Besseya bullii* (Eaton) Rydb. [Scrophulariaceae]) is a rare endemic species of sand-gravel prairies and savannas of the Midwestern United States (Gleason and Cronquist 1991), a region characterized by severe habitat destruction and fragmentation as a result of agriculture and urbanization. *S. bullii* is listed as endangered, threatened, or extirpated in all states across its range (Minnesota, Iowa, Wisconsin, Illinois, Indiana, Michigan, and Ohio [extirpated]). In addition, during the 1980's, this species was considered as a candidate for the US list of federally engendered and threatened plants, though listing was not granted (US Fish and Wildlife Service 1985). Although several reasons have been given for the decline of this species (e.g., trampling, crowding, quarrying), woody encroachment due to fire suppression is consistently listed as the primary reason for its decline.

In late April and early May, plants in Illinois populations produce one or more inflorescences with 10–130 flowers per spike. The flowers of *S. bullii* are hermaphroditic, dichogamous (i.e., temporal separation of pollen presentation [male phase] and stigma receptivity [female phase]), and protogynous (i.e., stigmas are receptive before pollen sheds), and the inflorescence flowers acropetally (i.e., maturing from the base towards the top; McKone et al. 1995). By late May or June, fruits (i.e., capsules) dehisce and small flat winged seeds are released. In terms of breeding system, previous studies have determined *S. bullii* to be self-compatible and pollinated by bees (McKone et al. 1995; Cholewa unpub. data). In our study sites, bees, in particular halictid bees (e.g., *Augochlorella*, *Augochlora*), *Xylocopa*, and *Bombus*, were confirmed to be the primary visitors of *S. bullii* inflorescences via pollinator behavioral observations (i.e., collection of pollen and/or contact of bee body parts with stigma). Lastly, McKone et al. (1995) noted that due to the dichogamous nature of *S. bullii* flowers in the inflorescence, pollinator behavior encourages the movement of outcrossing pollen.

Study sites and habitat classification

In April 2011, seven populations located in northwestern Illinois (USA) were selected for the study. In May, populations were classified according to canopy cover. Canopy cover was estimated based on the amount of sky that was visible when accounting for obstructions from nearby woody vegetation. Ten collection points were set up at a regular interval across the span of each population. At each collection point, the canopy was photographed using a tripod set at inflorescence height (i.e., ~14 ± 3 cm average across habitats) and Sigma SD14 SLR camera fitted with a fisheye lens. Photographs were imported into Canopy Analysis Software 2.1 to estimate the percentage of sky that was visible from the perspective of plants in the population. Populations were assigned to one of three habitat categories: open (visible sky: 92–100 %, $n = 2$), semi-shaded (visible sky: 57–80 %, $n = 2$), and shaded (visible sky: 10–38 %, $n = 3$). In addition, open habitats (i.e., LMF and LMR) were dominated by short, herbaceous vegetation and had no shading from an overhead woody canopy. Semi-shaded habitats (i.e., NAK and NAT) represented an intermediate category where herbaceous plants were still the dominant vegetation, but the *S. bullii* population occurred in at least partial shade as a result of one or two trees in the immediate area. Shaded habitats (i.e., FUL, PAM, and PAO) were areas where woody vegetation formed a closed canopy and very little sky was visible above the population. These shaded sites were historically open or semi-shaded. Also, populations were located between 30 and 80 miles apart, and when a location had more than one population, these populations were isolated by natural barriers. Lastly, all populations had more than 100 individuals.

Sampling

We used stigma pollen load (hereafter pollen load) as a proxy for pollinator visitation, as a relationship between pollinator activity and pollen load has been previously shown in other species (e.g., Engel and Irwin 2003). Measuring pollen quantity via pollen load was chosen as our tool for estimating pollinator activity because pollinator visitation at our sites was infrequent and difficult to accurately capture using only field observations. An exclusion experiment was used to determine pollen quality (self-pollen vs. cross-pollen) and rates of autonomous selfing. *S. bullii*'s breeding system has been studied and it has been found that plants do not differ in fruit set when dusted with self- versus outcross-pollen (McKone et al. 1995; Cholewa unpub. data). Thus, we chose not to repeat experiments on this plant's breeding system. Instead, we assumed that stigma pollen loads for excluded plants must originate exclusively from the same plant. Moreover, we assumed that any fruits or seeds that develop in the exclusion treatment are the products of self-pollen and reflect reproductive output in the absence of pollinators.

In April 2011, prior to flowering in each population, ten plants were haphazardly selected for a pollinators excluded treatment and another ten plants were selected for a open-pollinated treatment. Plants were selected by tossing a ball blindly within the population and tagging the nearest plant. To avoid potential confounding resource effects involved with producing multiple inflorescences, only those plants producing a single inflorescence were chosen.

Individuals in the pollinators excluded treatment were covered in mesh cages wrapped in a thin layer of bridal veil that prevented contact from insects or other plants, and only minimally interferes with light exposure (Kearns and Inouye 1993). Plants in the exclusion treatment were checked weekly to ensure that no part of the inflorescence contacted edges of the cage. Open-pollinated plants were marked with a tag but no other manipulation occurred.

From each plant in both treatments, three stigmas were haphazardly collected to determine pollen load per inflorescence. Stigmas were excised once female-phase flowers had fully transitioned to male-phase flowers and were no longer receptive, and stored in formalin–acetic acid–alcohol (FAA) at room temperature. To assess pollen load, stigmas were stained with a 20 % concentration aniline blue dye for 1 min. After being fixed on microscope slides with glycerin, stigmas were then examined under a transmitted light microscope using AxioVision 4.7 software and all visible pollen grains were counted. Because less than 10 % of the observed pollen was heterospecific in the open-pollinated plant treatment and 0 % in the pollinators excluded treatment (confirming effectiveness of the treatment), all of our analyses are focused on the conspecific pollen.

In June 2011, after fruit had formed, infructescences from the two treatments were collected from each population. The total number of fruit and unfertilized flowers was counted for each infructescence. Fruit set was calculated as the proportion of fruit that had successfully formed out of the total number of flowers in an inflorescence. For each infructescence, five fruit were haphazardly selected to determine a mean seed set. Seed set was calculated as the proportion of seeds that had formed out of the total number of ovules determined for *S. bullii* ($n = 52$).

Seed viability for the two treatments was determined through a germination study in a growth chamber. Seeds were removed from each of the collected infructescences. All seeds from each treatment at each population were mixed thoroughly to minimize effects of a single source plant on germination. A total of 80 seeds were then selected and separated into 4 petri dishes lined with moistened filter paper. For the cold stratification process, petri dishes were individually wrapped in aluminum foil and stored at 3 °C for 3 months. Afterward, petri dishes were unwrapped and placed in a growth chamber that simulated spring conditions (20 °C, 14-h photoperiod). Seeds were monitored daily for 4 weeks to check for signs of germination (i.e., emergence of roots and cotyledons). Lastly, seed germination conditions were based on preliminary studies (Chi unpub. data) and the need to avoid any concerns associated with dormancy.

Statistical analysis

Prior to analysis, all data were tested for normality by examining Q–Q Plots and calculating W values using the Shapiro–Wilk statistic with PROC UNIVARIATE in SAS software (SAS Institute Inc 2011). Data were considered normal when $W \geq 0.90$ and $p \geq 0.50$. To achieve a normal distribution, data for the pollinator exclusion experiment were transformed in the following ways: pollen data were log-transformed, and seed set and germination were transformed using $arcsin(\sqrt{x})$.

Regression analyses in PROC GLM were used to assess the relationship between pollen load and reproduction (i.e., fruit set and seed set) and pollen load and seed germination. In addition, a two-way factorial analysis followed by a Tukey's post hoc test in PROC GLM was used to examine differences among the pollinators exclusion treatment, habitat types and interaction of these factors for pollen load, fruit set, seed set, and seed germination. All statistical analyses were conducted using SAS (SAS Institute Inc 2011).

Results

When examining the relationship among pollen load, reproductive output, and germination patterns of association emerge for *S. bullii*. Pollen load, the number of pollen grains per load ranged from 0 to 192. Fruit set and seed set showed a positive trend with increasing pollen load, while germination appeared to have no relationship with pollen quantity (Fig. 1). Fruit set showed a significant positive relationship with pollen load ($R^2 = 0.659$, $p < 0.001$, $n = 14$). The additional pollen in the open-pollinated treatment led to increases in fruit set for all study populations, though the magnitude of increase varied among populations (Fig. 1a). For example, compared to the pollinators excluded

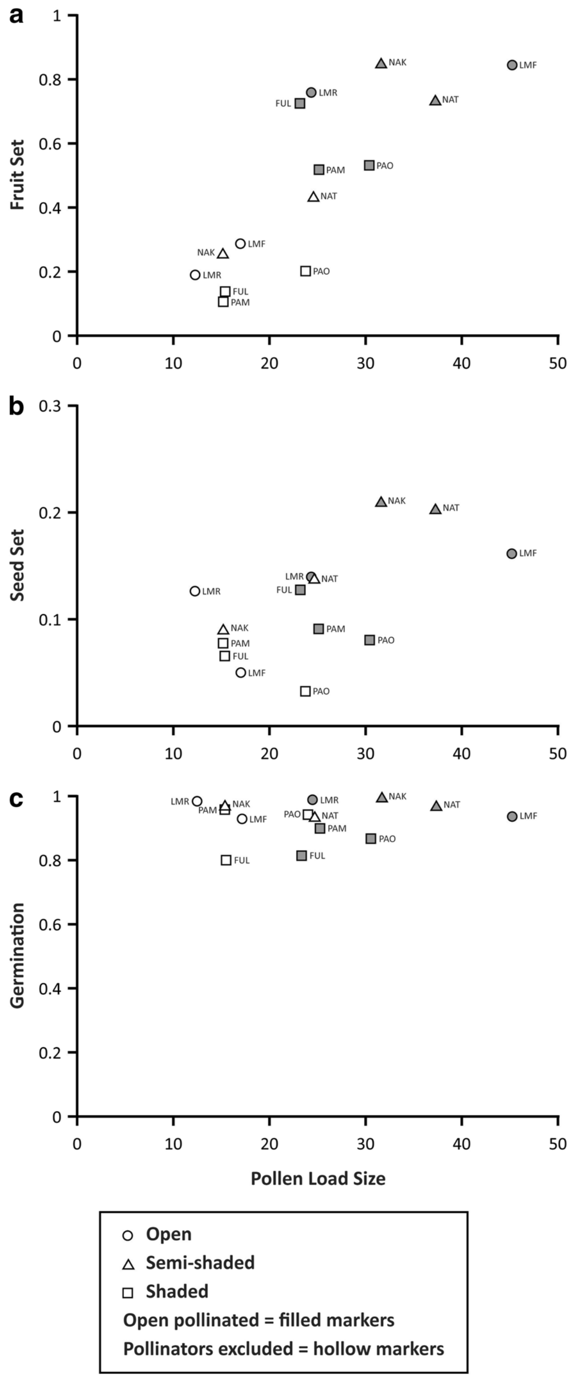

Fig. 1 Relationship between pollen load (i.e., mean number of pollen grains on stigma) and: **a** fruit set, **b** seed set, and **c** seed germination for *Synthyris bullii* populations

treatment, pollen load was higher in the control by 33 % in FUL and 34 % in NAT, and this led to an increase in fruit set of 82 and 41 %, respectively.

Similarly, seed set was found to increase with pollen load ($R^2 = 0.360, p = 0.023, n = 14$). As with fruit set, the magnitude of increase was not consistent among populations (Fig. 1b). Some populations showed a considerable increase in seed set when comparing the two treatments, such as FUL where a 33 % increase in pollen load in the open-pollinated versus pollinators excluded led to a 49 % increase in seed set. By comparison, a 49 % increase in pollen load in the open-pollinated treatment versus pollinators excluded for LMR corresponded with only a 9 % increase in seed set.

There was no relationship between germination and pollen load ($R^2 = 0.010$, $p = 0.733$, $n = 14$). The addition of pollen in the open-pollinated compared to pollinators excluded treatment did not result in an increase in seed germination (Fig. 1c).

The overall patterns described above are also influenced by the habitat type of the studied populations and the exclusion of pollinators. The pollinators excluded treatment was found to have a significant effect on pollen loads ($F = 27.41, \mathrm{df} = 1, p < 0.001$), but neither habitat ($F = 0.90, \mathrm{df} = 2, p = 0.414$) nor the interaction ($F = 0.90$, $\mathrm{df} = 2$, $p = 0.417$) were found to be significant. When pollinators were excluded from plants, there was a strong significant decrease in pollen load for all habitat types. Excluding pollinators reduced pollen load by approximately 57 % for open habitats, 49 % for semi-shaded habitats, and 32 % for shaded habitats. Although pollen load did not differ significantly among habitat types, there was on average 30 % fewer pollen grains on stigmas collected from open-pollinated plants in shaded habitats compared to open and semi-shaded ones (Fig. 2a).

Proportion of fruit set for plants ranged from 0.07 to 1.00. Significant differences were found for both main effects: pollinator exclusion treatment ($F = 207.63$, $\mathrm{df} = 1$, $p < 0.001$) and habitat ($F = 14.62$, $\mathrm{df} = 2$, $p < 0.001$); there was no significant interaction ($F = 1.10, \mathrm{df} = 2, p = 0.336$). In terms of treatment effect, pollinators excluded plants produced significantly lower fruit set compared to open-pollinated plants. Fruit set differed significantly among habitat types, with shaded habitats having lower fruit set than open and semi-shaded habitats (Fig. 2b).

The proportion of seed set ranged from 0 to 0.42. Both main effects (i.e., pollinator exclusion treatment [$F = 22.20, \mathrm{df} = 1, p < 0.001$] and habitat [$F = 8.41$,

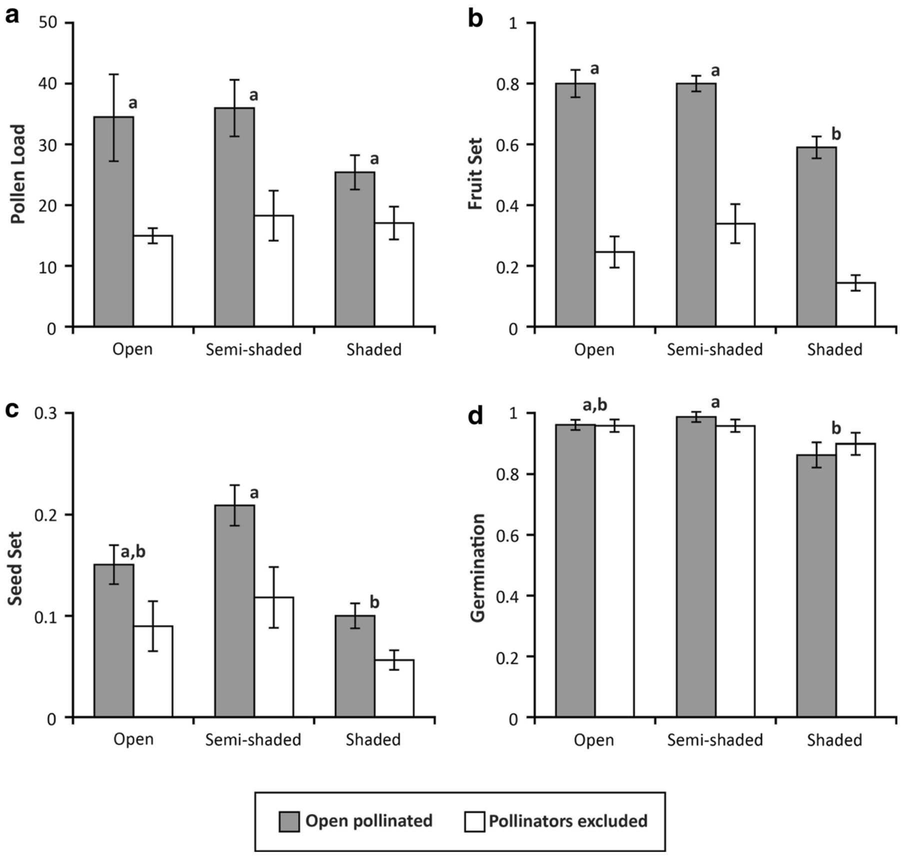

Fig. 2 Reproduction for *Synthyris bullii* in open-pollinated and pollinators excluded treatments for three habitats, showing means (±SEM) for **a** number of pollen grains on stigmas, **b** proportion of fruit set, **c** proportion of seed set, and **d** seed germination. Pollinators excluded treatment was found to significantly affect all reproductive variables except for germination. *Alphabetical letters* denote significant differences ($p \leq 0.05$) among habitat types

df = 2, $p < 0.001$]) were found to be significant, but there was no interaction between main effects ($F = 0.97$, df = 2, $p = 0.383$). Seed set was negatively affected by pollinator exclusion, resulting in lower seed set in pollinators excluded plants than open-pollinated plants. Seed set also differed significantly among habitat types. Shaded habitats showed significantly lower seed set than semi-shaded habitats, but not when compared to open habitats (Fig. 2c).

Seed germination for *S. bullii* was found to be relatively high, with a proportion of 0.55–1.00 seeds germinating successfully. Significant differences were found among habitats ($F = 4.90$, df = 2, $p = 0.011$), but not for treatment ($F = 0.01$, df = 1, $p = 0.913$) or the interaction ($F = 0.72$, df = 2, $p = 0.492$). Specifically, germination in semi-shaded habitats was significantly higher than in shaded habitats, while open habitats did not differ from either semi-shaded or shaded habitats (Fig. 2d).

Discussion

Habitat degradation such as woody encroachment has been identified as a factor that can lead to overall species decline in North American prairies and savannas (Ratajczak et al. 2012). In this study, we sought to assess how pollen quantity influences reproduction and seed germination and how changes in habitat structure as the result of woody encroachment influence these relationships in *S. bullii*, a rare species of prairies and savannas.

In the case of pollen quantity, we found fewer pollen grains on the stigmas of pollinators excluded plants compared to plants with pollinator access (Fig. 1). Plants without pollinator access generally had pollen loads ranging from 10 to 25 grains and a decreased fruit and seed set compared to open-pollinated plants, which had pollen loads ranging from 25 to 45 grains and increased fruit and seed set. In addition, our study found a positive relationship between pollen load and fruit/seed set. However, in the case of *S. bullii*, pollen quantity is unlikely to be the sole explanation for observed patterns in the reproductive success of our studied populations. When examining the relationship between stigma pollen load and fruit/seed set (Fig. 1a, b), some populations in the open-pollinated treatment (e.g., FUL) produced much higher fruit set even though pollen loads were small and comparable in quantity to pollen loads from the pollinators excluded treatment. Consequently, although autonomous selfing is occurring in this species, pollen quality (i.e., outcross-pollen) is also playing a role in the reproductive output of *S. bullii*.

Interestingly, while fruit and seed set are reduced by the exclusion of pollinators, seed germination was not affected. All seeds showed equal germinability, regardless of pollen quantity/quality. However, other studies have demonstrated that germination is artificially high under ideal laboratory conditions, and that fitness consequences only manifest in a natural field context (Dudash 1990; Ramsey and Vaughton 1998; Morgan 1999). Indeed, a study of *S. bullii* under different simulated environments resulted in variable seed germination (Curtis et al. 2013). In addition, while our study only examined seed viability, negative fitness consequences from selfing may still manifest at later life stages in terms of offspring survival and reproduction (Dudash 1990; Johnston 1992). While we found no germination differences in response to pollen quantity/quality, there may nevertheless be fitness consequences for traits that we failed to capture in this study.

The overall relationship among pollen quantity, reproduction, and seed germination is also influenced by the type of habitat in which *S. bullii* is found. Habitat conditions associated with woody encroachment may cause physiological stress or limit access to important resources (i.e., sunlight) (Van Auken and Bush 1997), which could lead to reduce reproduction. Because plants require light for both photosynthetic activity and as an environmental cue, shading can considerably alter the growth, development, and biomass allocation of herbaceous species (Slade and Hutchings 1987; Stuefer and Huber 1998). Differences in light availability and soil nutrient composition among habitat types may play an important role in terms of the resources available for reproduction functions. In addition, there is evidence that woody encroachment results in a decline of species richness in North American prairies and savannas (Ratajczak et al. 2012). Also, changes associated with woody encroachment have been shown to alter pollinator behavior. Several studies have demonstrated that ectothermic insect pollinators may avoid plants found in shaded areas (Herrera 1995; Culley 2002; Cortes-Palomec and Ballad 2006; Kilkenny and Galloway 2008).

While these abiotic factors associated with encroachment may have negative consequences for plant reproduction, in our study, decreased reproductive output in encroached areas involved reductions in pollen quantity and quality. Using pollen load as an indicator of pollinator activity, we determined that pollen quantity and quality is similar in open, semi-shaded, and shaded habitats (Fig. 2a). However, while the difference among habitat categories was found to be statistically non-significant, within the open treatment we still observed pollen loads that were 30 % smaller on average in shaded habitats relative to the other two habitats, suggesting that pollen quantity is lower in shaded areas. Our results support the notion that encroachment has negative impacts on pollinator visitation for *S. bullii*. As woody encroachment changes habitat quality and structure, the optimal pollinators associated with these plant species may be lost (Potts et al. 2010) and/or change their foraging behavior in response to the microclimatic conditions associated with increased shading (Kilkenny and Galloway 2008). This in turn may negatively affect plant–pollinator relationships and the reproductive success of the plants.

In addition, when comparing the pollen load of open-pollinated and pollinators excluded plants, it is apparent that self-pollen can make up as much as 68 % of total pollen loads of natural plants in shaded habitats, suggesting that pollinators in encroached areas are making somewhat limited contributions toward pollen deposition. The high proportion of self-pollen in the pollen loads of encroached areas is of particular concern because self-pollen can contribute to reduced reproduction even in self-compatible species (Aizen and Harder 2007). Indeed, when comparing the open-pollinated treatment for all habitats, fruit set and seed set for shaded populations were lower than the other two habitat categories (Fig. 2b, c). Taken together, this shows that the reduced reproductive output observed in encroached areas may be caused by a combination of reduced pollinator activity and increased fertilization by self-pollen. It is of interest, however, that although differences were found in seed germination among habitats, results from the pollinators excluded treatment suggest that pollen quality does not seem to be a factor in the ability of seeds to germinate, at least in a growth chamber.

In conclusion, while other studies have established patterns of species decline following woody encroachment (Rejmánek and Rosén 1988; Ratajczak et al. 2012), the research presented here suggests habitat degradation disrupts plant–pollinator interactions in a way that could compromise the viability of populations of rare plant species. Management of prairies and savannas via prescribed burns and/or mechanical removal of woody vegetation must be addressed if we want to aid in the recovery and maintenance of rare plant populations, plant–pollinator relationships, and overall diversity.

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